

IN THE CLAIMS

1-13 (canceled)

14. (currently amended) A method for ~~identifying and validating the effect of a candidate gene that is expressed in a mammalian neural cell of interest~~ an active double-stranded RNA (dsRNA) which attenuates a desired gene expression in a cell, said method comprising:

- (a) producing a candidate dsRNA which comprises at least ~~a portion~~ 100 nucleotides of a ~~said candidate gene that is expressed in a control cell~~;
- (b) introducing the said candidate dsRNA into a reference mammalian neural cell; and
- (c) ~~identifying whether the candidate dsRNA is an active dsRNA~~ validating the effect of said candidate gene by detecting an alteration in a cellular activity or a cellular state in said the reference mammalian neural cell, wherein said alteration is the result of specific attenuation of mRNA corresponding to said candidate in said reference mammalian neural cell, indicating that said the candidate gene plays a functional role in ~~the reference cell and is an active dsRNA~~ mammalian neural cells.

15. (currently amended) The method of claim 14, wherein said step of producing the candidate dsRNA comprises: (i)

producing a cDNA corresponding to said candidate gene from an mRNA of the control cell such that the cDNA comprises at least a portion of the gene that is expressed in the control cell said mammalian neural cell of interest; and (ii) ~~producing the candidate dsRNA from at least one of the said cDNA of said step (i).~~

16. (canceled)

17. (currently amended) The method according to Claim 15, further comprising:

~~A method for correlating genes and gene function, said method comprising: (a) producing a plurality of candidate dsRNAs from a plurality of cDNAs from said mammalian neural cell of interest of a control cell such that each candidate dsRNA comprises at least a portion of a gene that is expressed in the control cell; (b)~~

producing a plurality of candidate dsRNA which comprise at least 100 nucleotides of said candidate cDNAs;

introducing each of the candidate dsRNA into a plurality of separate reference mammalian neural cells each having a gene expression similar to said mammalian neural cell of interest the ~~control cell in step (a);~~

~~and (e) identifying which candidate dsRNA is an active dsRNA by detecting an alteration in a cellular activity or a cellular state in the reference cell, desired alteration indicating that the gene corresponding to the candidate dsRNA plays a functional role in the reference cell~~ validating the effect of said candidate genes by testing for alterations in a cellular activity or a cellular state in said reference mammalian neural cell that result of attenuation of mRNA corresponding to said candidate in said reference mammalian neural cell, wherein detection of said alterations is indicative that said candidate gene plays a functional role in said mammalian neural cells of interest.

18. (canceled)

19. (currently amended) The method of claim ~~18~~ 17, wherein said step of producing a plurality of candidate cDNAs comprises: (i)

~~isolating at least one mRNA from the cell;~~ (ii) producing a double-stranded cDNA from the ~~isolated~~ mRNA by reverse transcription; (iii)

producing cDNAs of a similar length by digesting said cDNA of said step (ii) with a restriction enzyme; and ~~(iv)~~

producing a plasmid or PCR fragment from said the cDNA of said step (iii) after said digesting step.

20. (currently amended) The method of claim 19, wherein the candidate dsRNA is produced by transcribing the said plasmid cDNA or PCR fragment ~~of said step (iv).~~

21. (canceled)

22. (original) The method of claim 19, wherein the restriction enzyme is selected from the group consisting of Dpn1 and Rsa1.

23. (currently amended) The method of claim 17, wherein said step of producing the plurality of candidate dsRNAs comprises: ~~(A) selecting a candidate cDNA gene, wherein the candidate gene is a gene that is expressed in a test cell and/or a control cell, and/or that is expressed at a detectably different level with respect to the~~ said reference mammalian neural cell and said

mammalian neural cell of interest test cell and the control cell, and said reference mammalian neural cell and said mammalian neural cell of interest ~~the test cell and control cell~~ differ with respect to a cellular characteristic that is detectable by said step of testing for alterations in a cellular activity or a cellular state; and ~~(B) producing the plurality of candidate dsRNAs, wherein each candidate dsRNA is substantially identical to at least a part of the candidate gene.~~

24. (currently amended) The method of claim 23, wherein the candidate cDNA gene is selected from a normalized library prepared from said reference mammalian neural cells or said mammalian neural cell of interest ~~cells of the same type as the test cell or the control cell~~ and is present in low abundance in the normalized library.

25. (currently amended) The method of claim 23, wherein the candidate cDNA gene is a differentially expressed cDNA gene selected from a subtracted library that is enriched for cDNAs genes that are differentially expressed with respect to said reference mammalian neural cells or said mammalian neural cell of interest ~~the test cell and the control cell~~.

26. (canceled)

27. (currently amended) The method of claim 23, wherein said step of selecting the candidate cDNA gene comprises: ~~(i)~~

preparing ~~(A)~~ a tester-normalized cDNA library ~~which is a normalized library prepared from test cells~~; ~~(B)~~ a driver-normalized cDNA library ~~which is a normalized library prepared from control cells~~; ~~(C)~~ a tester-subtracted cDNA library which is enriched in one or more genes that are up-regulated with respect to the test cell and the control cell, and ~~(D)~~ a driver-subtracted cDNA library which is enriched in one or more genes that are down-regulated with respect to the test cell and the control cell; and ~~(ii)~~

~~identifying one or more clones~~ selecting a cDNA from the normalized libraries ~~and/or the subtracted libraries, wherein the candidate gene is one of the clones identified by~~ ~~(A)~~ contacting clones cDNAs from the tester-normalized cDNA library with labeled probes derived from mRNA from test cells and contacting clones cDNAs from the driver-normalized cDNA library with labeled probes derived from mRNA from control cells under conditions whereby probes specifically hybridize with complementary clones cDNAs to form a first set of hybridization complexes; and ~~(B)~~ detecting at least one hybridization complex from the first set of hybridization complexes to identify a ~~clone from one of the normalized libraries which~~ cDNA ~~that~~ is present in low abundance.

28. (canceled)

29. (currently amended) ~~The method of claim 27, wherein said step of identifying one or more clones~~ The method of claim 23, wherein said step of selecting the candidate cDNA comprises:
preparing a tester-normalized cDNA library from test cells; a driver-normalized cDNA library from control cells; a tester-subtracted cDNA library which is enriched in one or more genes that are up-regulated with respect to the test cell and the control cell, and a driver-subtracted cDNA library which is enriched in one or more genes that are down-regulated with respect to the test cell and the control cell; and

selecting a cDNA from the subtracted libraries comprises: (A) by contacting clones cDNAs from the tester-subtracted cDNA library and contacting clones cDNAs from the driver-subtracted cDNA library with a population of labeled probes under conditions whereby probes from the population of probes specifically hybridize with complementary clones cDNAs to form a second set of hybridization complexes, and wherein the population of labeled probes is derived from mRNA from test cells and control cells; and (B) detecting at least one hybridization complex from the second set of hybridization complexes to identify a clone cDNA from one of the subtracted libraries which that is differentially expressed above a threshold level with respect to the subtracted libraries.

30. (currently amended) The method of claim 23, wherein the cellular characteristic is cell health, the test cell is a diseased neural cell and the control cell is a healthy neural cell, and the candidate gene is ~~potentially correlated~~ suspected of correlation with a disease.

31. (original) The method of claim 30, wherein the test cell is obtained from a mammal that has had a stroke or is at risk for stroke.

32-33. (canceled)

34. (currently amended) The method of claim 23, wherein the cellular characteristic is cellular differentiation and the candidate gene is ~~potentially correlated~~ suspected of correlation with ~~controlling~~ control of cellular differentiation.

35. (currently amended) The method of claim 23, wherein the candidate gene is an endogenous ~~gene of the~~ to said mammalian neural reference cell.

36. (currently amended) The method of claim 23, wherein the candidate gene is ~~present in the reference cell as~~ an extrachromosomal gene in said mammalian neural reference cell.

37-42 (canceled)

43. (currently amended) The method of claim 30 42, wherein ~~the~~ said mammalian neural reference cell is a neuroblastoma cell.

44. (canceled)

45. (currently amended) The method of claim 44, wherein ~~the~~ said mammalian neural reference cell has increased sensitivity to N-methyl-D-aspartate, ~~beta~~ β -amyloid, peroxide, oxygen-glucose ~~glucose~~ deprivation, or combinations thereof, relative to a normal mammalian neural cell.

46. (currently amended) The method of claim 45, wherein the detecting step comprises detecting a decrease in cellular sensitivity to N-methyl-D-aspartate, ~~beta~~ β -amyloid, peroxide, oxygen-glucose ~~glucose~~ deprivation, or combinations thereof, relative to a normal mammalian neural cell.

47. (original) The method of claim 17, wherein the detecting step comprises detecting modulation of ligand binding to a protein.

48-50 (canceled)

51. (amended) The method of claim 14 47, wherein the determining step comprises determining whether the protein encoded by the candidate gene binds to another protein to form a coimmunoprecipitating complex ~~that can be coimmunoprecipitated~~.

Add the following new claims:

52. (new) The method of claim 14, wherein the candidate dsRNA is at least 500 nucleotides in length.

53. (new) The method of claim 14, wherein the candidate dsRNA is between 500 and 1100 nucleotides in length.

54. (new) The method of claim 14, wherein said mammalian neural cell of interest is a glial cell.

55. (new) The method of claim 14, wherein said reference mammalian neural cell is a glial cell.